SUPPORT FOR THE AMENDMENTS

Claims 1, 2, and 12-14 have been amended.

Claims 4 and 25-30 have been canceled.

The amendment of Claims 1, 2, and 12-14 is supported by the original Claims 1-3 and pages 7-8 of the specification, for example at page 7, lines 3-14 and the Examples.

No new matter is believed to have been entered by the present amendments.

REMARKS

Claims 1-3 and 5-24 are pending in the present application.

The rejections of: (a) Claims 1-9, 12-16, 20-22, 24, 26-28, and 30 under 35 U.S.C. §102(b) over LaFrentz et al; and (b) Claims 1-8, 12-15, 20-22, 24, 26-28, and 30 under 35 U.S.C. §102(b) over Masunari et al, are respectfully traversed.

Applicants submit that the Examiner's rejections over LaFrentz et al and Masunari et al are without merit and overlook a critical element of the claimed invention. Specifically, neither of LaFrentz et al and Masunari et al disclose inactivation of a logarithmic growth phase culture.

The rejections over LaFrentz et al and Masunari et al are again maintained for the same basic reason. The Examiner's basic position can be summarized as "logarithmic phase is defined as the phase where binary fission occurs and the rate of increase in cell number is multiplication function of cell number. The culture conditions of [LaFrentz et al or Masunari et al] are such that the cells would be in logarithmic phase." The Examiner then alleges that the burden is shifted to Applicants to prove that the conditions disclosed in LaFrentz et al and Masunari et al would not be in logarithmic phase. Applicants disagree with the Examiner's conclusions.

In an attempt to support the alleged conclusions, the Examiner points to the sentence bridging pages 704 and 705 of LaFrentz et al, which disclose growing *F. psychrophilum* in 2L of tryptone yeast extract salt (TYES) broth at 15°C for 72 hours. It is the Examiner's position that such conditions would inherently be in logarithmic phase.

Similarly, the Examiner alleges that Masunari et al discloses a logarithmic growth phase in the growth curve in Figure 1, referencing page 814. However, this indication by the

Examiner makes no sense as Figure 1 of Masunari et al makes no reference to logarithmic growth phase and does not contain a growth curve. Further, the Examiner refers to page 814, which does not exist either in the Japanese text of Masunari et al or the English translation.

Applicants again submit that Masunari et al disclose culturing *F. psychorophilum* for 3 to 3.5 days in modified cytophaga broth at 18°C. LaFrentz et al disclose culturing *F. psychorophilum* for 72 hours in TYES at 15°C. Not withstanding the fact that Masunari et al disclose that the amount of live cells before inactivation was 10^6 CFU/ml, Applicants submit that based the similarities in the growth conditions, the growth curve of *F. psychorophilum* shown in Figure 1 of the present application, and the disclosure at page 9, lines 11-15, it is clear that neither Masunari et al, nor LaFrentz et al disclose inactivation of a logarithmic growth phase culture.

Although the Examiner describes a definition of a logarithmic growth phase as increasing in cell number and alleges that the growth conditions in Masunari et al and LaFrentz et al meet this definition, there is no disclosure in the references to show such an increase in cell number during their growth conditions as the Examiner alleges. Indeed, LaFrentz et al and Masunari et al disclose an incubation period for more than 3 days and 72 hours, respectively.

Based on the growth conditions reported in these references, Applicants submit that the culture would not be in logarithmic phase as the Examiner alleges, but rather they would be in the stationary phase. It is a well known fact that the stationary phase is a time of significant physiological change and particularly involves the physiological adaptation of cells to survival through periods of little growth. Frequently these physiological changes are manifest in altered physical structure (e.g., membrane) or differences in protein expression profiles.

Moreover, as shown in Example 4 (Table 1 on page 12 of the specification; reproduced below for the Examiner's convenience), vaccines made from a culture in logarithmic phase (i.e., the present invention) have a higher efficacy than those in stationary phase (i.e., LaFrentz et al and Masunari et al).

TABLE 1

Group	Dosage of Challenge (CFU/mL)	Death/ Challenge	Survival Rate (%)
Logarithmic Growth Phase Group	1.7×10^8	39/152	74[a,c]
Stationary Phase Group	1.9×10^8	39/105	63[b]
Control Group	2.2×10^8	82/165	50

a: Significant difference against control group ($p < 0.001$), chi-square test

b: Significant difference against control group ($p < 0.05$)

c: Significant difference against stationary phase group ($p < 0.05$)

In view of the foregoing, Applicants submit that the term "logarithmic phase" sufficiently characterizes and distinguishes the present invention from the disclosures of LaFrentz et al and Masunari et al. Therefore, none of these references can anticipate the claimed invention.

With respect to Applicants pointing out that the culture referred to by LaFrentz et al and Masunari et al is not a "logarithmic phase" but rather is in "stationary phase", the Examiner disregards the same alleging "Attorney argument." Applicants disagree that this is "Attorney argument". Instead, the foregoing points of the facts supported by the disclosures of LaFrentz et al and Masunari et al. To further evidence the same, Applicants **submit herewith** an executed Declaration under 37 C.F.R. §1.132 ("the Declaration") evidencing that the cultures in the art of record are, in fact, "stationary phase".

In paragraph (8) of the Declaration, it is stated that:

> In this experiment, we studied proliferating potential of the bacteria in modified cytophaga (Masunari et al's), and found that the culture reached stationary phase within about 24 to 36 hrs. As stated in paragraph (6), above, in LeFrentz et al, the bacteria were cultured in TYES medium at 15°C for **72 hours**; in Masunari et al, the bacteria were cultured in modified cytophaga medium at 18°C for 3 to 3.5 days (i.e., **72-90 hours**); and, in Rahman et al, the bacteria were cultured in MCY (modified cytaphaga) medium at 20°C for 48 hrs and in COY medium at 20°G for 24 hrs (totally for **72 hours**). Thus, the result of the experiments herein and illustrated in the figures appearing in paragraph (7) establish that bacteria used in LeFrentz et al, Masunari et al, and Rahman et al were in stationary phase, rather than logarithmic phase as those used in the present invention.
>
> Moreover, as the culture conditions of the cited references were not clearly disclosed, the skilled artisan would not expect the advantageous effect of the bacteria in logarithmic phase as used in the present invention from the description of the references.

In view of the Declaration enclosed, specifically the evidence provided in paragraphs (7) and (8), Applicants submit that neither of LaFrentz et al and Masunari et al disclose inactivation of a logarithmic growth phase culture as required in the claimed invention.

Accordingly, Applicants request withdrawal of these grounds of rejection.

The rejection of Claims 1-8, 12-15, 20-22, 24, 26-28, and 30 under 35 U.S.C. §102(b) over Rahman et al is respectfully traversed.

The Examiner continues to maintain this rejection over Rahman et al. The Examiner again refers to the section entitled "Culture Conditions in Broth Medium" on page 173 as disclosing harvesting of cells in logarithmic growth phase. However, there is no disclosure in Rahman et al that the logarithmic growth phase cells were inactivated. The Examiner points to the disclosure on page 170 and alleges that "formalin killed bacteria was used for the vaccine in question." This may be the case, but this disclosure relates to the colonies that were harvested by *scraping* from CGY agar plates as discussed in the section entitled

"Bacterial Strain and Culture Conditions on Agar" appearing on page 170, not the logarithmic growth phase culture from page173.

The Examiner also references the *F. psychorophilum* vaccine based on the antigenic outer membrane fraction (OMF vaccine) of the cell. However, contrary to the Examiner's assertion, the OMF vaccine was not prepared by inactivation with formalin (see method disclosed on page 171, first full paragraph). In fact, on pages 172-173 and the Results section, Rahman et al clearly focus on the differences between the formalin-killed bacteria (FKB) and the OMF antigens. Accordingly, the Examiner's rejection over Rahman et al is without merit.

Moreover, even if the artisan were to refer to the section referring to the culture conditions of *F. psychrophilum* on page 170, the bacteria were cultured in MCY (modified cytaphaga) medium at 20°C for 48 hrs and in CGY medium at 20°G for 24 hrs (totally for 72 hours). As shown in the Declaration, after 72 hours a culture of *F. psychrophilum* is in stationary phase, not logarithmic phase as required by the present invention.

In view of the foregoing, Applicants submit that the presently claimed invention is not anticipated by Rahman et al. Withdrawal of this ground of rejection is requested.

The rejections Claims 3, 4, and 19-30 under 35 U.S.C. §112, first paragraph (written description and enablement), are obviated in part by amendment and traversed in part.

Applicants make no statement with respect to the propriety of this ground of rejection over Claims 4 and 25-30, other than to disagree with the assertions and conclusions by the Examiner. However, to expedite examination of the remaining claims, Claims 4 and 25-30 have been canceled without prejudice toward their presentation in an ensuing application.

With respect to Claims 3 and 19-24, the Examiner has rejected these method claims as lacking a sufficient written description and/or enabling support. The Examiner position

appears to be two-fold: (a) the scope of the inactivated whole cell or components thereof is not adequately described and enabled so as to correlate the same to the effect indicated, and (b) the specification allegedly fails to provide substantive evidence that the claimed method is capable of inducing protective immunity against cold-water disease as claimed.

With respect to (a), the Examiner real criticism appears to be that it is unclear which "components" will convey the protective response alleged. Applicants submit that the cancellation of Claim 4 and the amendment to Claim 2 to insert the term "and" in the place of "and/or" remedies this concern. Specifically, the presently pending method claims relate to the protective immune response elicited by inactivated *whole* cells of *F. psychrophilum* in a logarithmic growth phase. Thus, this criticism by the Examiner is moot.

As for (b), Applicants submit that the specification and examples clearly provide direct evidence of the protective immune response elicited by inactivated *whole* cells of *F. psychrophilum* in a logarithmic growth phase. Specifically, Examples 2 and 3 describe the preparation of the inactivated cells, while Examples 4 and 5 clearly show challenge experiments and the resulting protective immune response, which the Examiner alleges are not provided. On the basis of these Examples, Applicants traverse the Examiner's written description and enablement rejections for the inactivated *whole* cells of *F. psychrophilum* in a logarithmic growth phase.

Further, Applicants submit that the evidence provided by Examples 2-5 to support enablement of the claimed method using inactivated *whole* cells of *F. psychrophilum* in a logarithmic growth phase is reasonably correlated to the scope of the claimed invention. As such, the skilled artisan can readily appreciate how to make and use the invention as claimed.

Accordingly, Applicants request withdrawal of these grounds of rejection.

The rejection of Claims 1-30 under 35 U.S.C. §112, second paragraph, is obviated by amendment.

In making this rejection, the Examiner appears to have two criticisms:

1) The term "inactivated" is not defined and is unclear. The Examiner further states that it "if cell are not alive, they cannot be in a log phase" and the Examiner questions "what core features/structures must be maintained". And,

2) The phrase "wherein said *Flavobacterium psychrophilum* in a lograrithmic growth phase are inactivated by formalin treatment" appearing in Claim 14 lacks antecedent basis in Claim 2, since this claim is drawn to components of inactivated cells.

Applicants submit that the term "inactivated" is clear to the skilled artisan based on the general knowledge available in the art and the description in the specification. For example, in the last line on page 169 of Rahman et al, the authors use the same language and phrasing as in the present application (i.e., "inactivated whole cell *F. psychrophilum*..."). Further, at page 7, lines 6-7 of the present specification, as well as in Examples 2-3, Applicants provide exemplary inactivation treatments. With respect to the core features/structure to be maintained, it is our opinion that page 7, lines 8-9 of the specification make it clear that the core features/structure to be maintained is membrane components, vesicles, and secretary products. As such, no amendment is necessary. Nonetheless, to avoid any further delay in examination of this application, Applicants have amended Claims 1, 2, and 12-14 to address the Examiner's criticisms. Specifically, as exemplified by Claim 1, Claims 1, 2, and 12-14 have been amended as follows:

> 1. (Currently Amended) A pharmaceutically administrable composition comprising inactivated cells of *Flavobacterium psychrophilum* cultivated from ~~in~~ a logarithmic growth phase culture and at least one pharmaceutically acceptable carrier or adjuvant.

With respect to the criticism of Claim 14, this claim has been amended to ensure antecedent basis for all terms are present. Note, Claim 13 has been similarly amended.

In view of the amendments herein, Applicants request withdrawal of this ground of rejection.

Applicants submit that the application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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